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INCORPORATION OF *Boletus edulis* EXTRACT IN BEEF BURGERS PATTIES: PRELIMINARY EVALUATION OF THE ANTIOXIDANT PROTECTIVE EFFECTS

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KEYWORDS

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ABSTRACT

The objective of this study was to analyse the antioxidant protective effects of the incorporation of *Boletus edulis* extract in beef burgers. The relationship of total lipids/fatty acids to lipid peroxidation of hamburger during storage was examined. Beef burgers samples (lipids content: 15–18%) were stored for 8 days, and thiobarbituric acid reactive substances (TBARS) formation inhibition, free radical scavenging activity and reducing power were determined. The antioxidant potential increased with the amount of extract added to beef burgers, giving higher radical scavenging properties and TBARS inhibition capacity. These findings indicated that *Boletus edulis* extract had a protective antioxidant effect against lipid peroxidation of beef burgers patties.

INTRODUCTION

Lipid peroxidation is a major quality deterioration problem in foods. The primary products of the reaction are lipid hydroperoxides, and they are converted to secondary products such as aldehydes. The development of warmed-over flavor, which is caused by aldehydes, can be accelerated by lipid peroxidation in raw meats during storage. Lipid peroxidation products are also considered as compounds harmful to human health. It has been reported that oral intake of fatty acid autooxidation products stimulated lipid peroxidation in living organs. Secondary products of lipid peroxidation such as malonaldehyde and 4-hydroxynonenal are cytotoxic and mutagenic compounds [1]. Thus, lipid stability is one of the important factors for maintaining meat quality during storage. Several antioxidants are used to prevent lipid oxidations in animal meats.

Different mushroom species were reported to have antioxidant activity, which was mainly related to their phenolic content [2]. Moreover we have reported the antioxidant properties including free radical scavenging

and lipid peroxidation inhibition properties of *Boletus edulis* methanolic extracts [3]. The objective of the present study was to evaluate the antioxidant protective effects of this mushroom extract when incorporated in beef burgers patties during storage.

MATERIALS AND METHODS

Samples and sample preparation

Boletus edulis Fr. was a commercial dried sample obtained in a local supermarket. The sample was extracted with aqueous acetone 70% (v/v) following the procedure described by Bao et al. [4]. The mushroom extract was added separately to each portion of beef burgers to give a final concentration of 5, 1 and 0% (control). Each sample was thoroughly blended, and a 100 g portion of the hamburgers was packed individually and subsequently stored at 4°C for 8 days.

pH Measurement

The pH was measured after homogenization of the samples (~3g) with distilled water, centrifugation and filtration.

Determination of total lipids and fatty acid composition

Total lipids were extracted from lyophilized sample in a Soxhlet apparatus, and determined gravimetrically following AOAC procedure. The fatty acid profile was analysed, after a trans-esterification procedure, with a DANI model GC 1000 instrument equipped with a split/splitless injector, a FID and a Macherey-Nagel column (30 m × 0.32 mm ID × 0.25 µm *df*). The oven temperature program was as follows: the initial temperature of the column was 50 °C, held for 2 min, then a 10 °C/min ramp to 240 °C and held for 11 min. The carrier gas (hydrogen) flow-rate was 4.0 ml/min (0.61 bar), measured at 50 °C. Split injection (1:40) was carried out at 250 °C.

Evaluation of antioxidant activity

The lyophilized samples were extracted with methanol (1h+1h) and submitted to three different antioxidant activity evaluation assays. The results were expressed in EC₅₀ values (extract concentration necessary to achieve 50% of antioxidant activity).

DPPH radical-scavenging activity

This methodology was performed using an ELX800 Microplate Reader (Bio-Tek Instruments, Inc). The reaction mixture in each one of the 96-wells consisted of extract solution and aqueous methanolic solution containing DPPH radicals. The mixture was left to stand for 60 min in the dark. The reduction of the DPPH radical was determined by measuring the absorption at 515 nm.

Reducing power

This methodology was performed using the Microplate Reader described above. The presence of reducers (i.e. antioxidants) causes the conversion of the Fe³⁺/ferricyanide complex used in this method to the ferrous form. Therefore, by measuring the formation of Perl's Prussian blue at 700 nm we can monitor the Fe²⁺ concentration; a higher absorbance at 700 nm indicates a higher reducing power (RP).

Inhibition of lipid peroxidation using thiobarbituric acid reactive substances (TBARS)

It was measured the colour intensity of the malondialdehyde (MDA)-TBA complex formed in the system by its absorbance at 532 nm [3].

Statistical analysis

Data were submitted to analysis of variance using the R Development Core Team (2008) software. A factorial design of 2×3×3 was used, and treatment means were compared using the multiple comparisons Fisher's least significant difference with a significance level of 5%.

RESULTS AND DISCUSSION

The effect of storage time and *Boletus edulis* extract concentration in pH and EC₅₀ values of DPPH, RP and TBARS of the beef burgers are shown in Table 1. The beef burgers initial total lipids content was 17.7 g/100 g. The pH and EC₅₀ values of DPPH and TBARS decreased ($P < 0.05$) with the increase of beef burgers storage time. This trend was also observed for RP EC₅₀ values, however the effect it wasn't statistically significant ($P > 0.05$). The pH and EC₅₀ values of DPPH, RP and TBARS decreased ($P < 0.05$) with the increase of *Boletus edulis* extract concentration. These results show that after one day of storage the beef burgers are already subjected to fat

oxidation. The addition of the mushroom extract increased the antioxidant potential (decreasing of EC₅₀ values) and the reducing power of beef burgers patties. On contrary, the addition of mushroom extract inhibits the formation of TBARS on beef burgers patties. The treatment with *Boletus edulis* extract promoted lower oxidation index, and the fatty acids were remarkably protected from oxidation after 8 days of beef burgers patties storage (data not shown).

Table 1: Effect of storage time (days) and *Boletus edulis* extract concentrations (%) in pH and EC₅₀ values of DPPH (mg/ml), RP (mg/ml), and TBARS (mg/ml) of beef burgers

	n	pH	DPPH	RP	TBARS
Storage, days					
1	9	5.7±0.02 ^a	105±30.4 ^a	3.7±0.49 ^a	20.6±5.41 ^a
8	9	5.1±0.05 ^b	5.1±0.05 ^b	3.3±0.43 ^a	12.8±3.70 ^b
[Extract], %					
0	6	5.5±0.10 ^a	147±35.7 ^a	5.1±0.46 ^a	34.6±3.31 ^a
1	6	5.4±0.17 ^a	44±1.63 ^b	3.1±0.23 ^b	10.0±1.60 ^b
5	6	5.3±0.17 ^b	35±2.80 ^b	2.4±0.12 ^b	5.5±0.39 ^c
Main effects					
Storage		***	***	NS	***
[Extract]		***	***	***	***
Storage*[Extract]		***	***	NS	***

Different letters within columns indicate significant ($P < 0.05$) differences between mean values; NS = non significant ($P > 0.05$); *** $P < 0.001$.

The interactions between *Boletus edulis* extract concentration and storage time effects on pH and EC₅₀ values of DPPH and TBARS are shown in Figure 1. The interactions were highly significant for pH and EC₅₀ values of DPPH and TBARS. It is remarkable that antioxidant capacity of the samples increased with the increase of mushroom extract concentration. Nevertheless, the same behaviour was observed in control samples, with a reduction in pH and EC₅₀ values of DPPH and TBARS with the storage time. The activity of some antioxidant defences (non-enzymatic or enzymatic) present in beef burgers is increasing along the storage time, in response to the oxidative stress inherent to the storage process. Thus, further work is necessary to elucidate the interactions herein reported.

CONCLUSIONS

Based on the study results, it is noteworthy to point out that *Boletus edulis* extract had a protective effect in beef burgers against lipid peroxidation. The addition of mushroom extract can be used to extend the beef burgers shelf-life during storage. Hydrophilic extract prepared from *Boletus edulis* is a promising source of natural antioxidants for food and food stuffs.

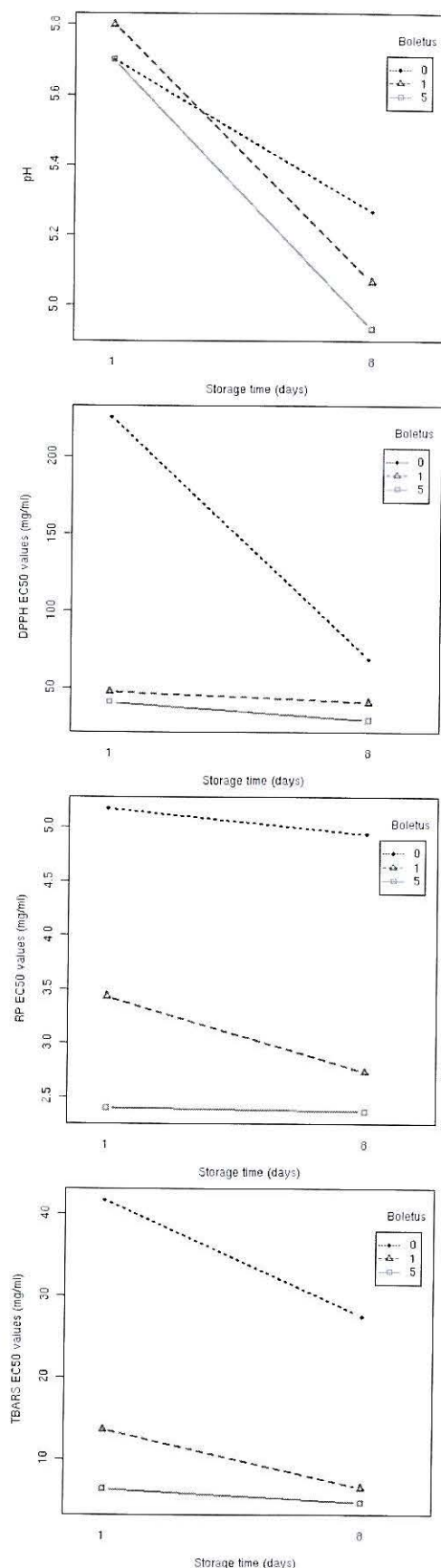


Figure 1: Interactions between *Boletus edulis* extract concentration and storage time effects on beef burgers patties for pH and EC₅₀ values of DPPH (mg/ml), RP (mg/ml) and TBARS (mg/ml)

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